

HOW TO DETECT WHEN CELLS IN SPACE PERCEIVE GRAVITY

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It is useful to be able to measure when and whether cells detect gravity during spaceflights. For studying gravitational physiology, gravity perception is the response the experimentalist needs to measure. Also, for growing plants in space, plant cells may have a non-directional requirement for gravity as a developmental cue.

GRAVITATIONAL PHYSIOLOGY

The main goals of spaceflight experiments in which gravity perception would be measured are to determine the properties of the gravity receptor and how it is activated, and to determine fundamental characteristics of the signal generated.

Measuring gravitropic curvature. The main practical difficulty with measuring gravity sensing in space is that we cannot measure gravity sensing with certainty on earth. Almost all experiments measure gravitropic curvature. Gravitropic curvature is measurable only when growth and growth regulation are functioning normally. This may not be the case in a space experiment and it certainly is not the case in cell culture.

Because the many physiological processes between perception and curvature can be influenced by environmental factors, particularly the gaseous environment, the conditions in which the experiments are done are particularly critical for experiments using gravitropic curvature as the assay.

Gravitropic variants and mutants have been hopefully used to learn about the mechanism of gravitropism, yet all seem to differ in transduction or response, not perception. That suggests that gravity perception is so fundamental that it is very rarely absent. It also means that gravitropic variants have been less helpful than expected in learning about gravity perception per se.

Gravitropic bending can be used to make inferences about gravity perception when appropriately used. Presentation-time measurements give a dose-response curve for perception with the response allowed to go to completion. By varying the gravitational force, the reciprocity between time and force can be used to test whether perception is a function of sedimentation (Johnsson, 1965). Intermittent stimulation with varied lengths of stimulation and intermission can give information about time averaging (e.g. sampling period), memory, and signal to noise ratio of the gravity receptor.

Reciprocity and intermittent stimulation are measurements which have been made to some degree on earth using clinostatting, but which would provide clearer results if done with microgravity rather than clinostatting. These would be important uses of the space laboratory for determining the nature of gravity sensing in plants.

Electrical measures of gravity sensing. Those techniques which do not use gravitropic curvature to measure gravity sensing are electrophysiological. These are based on phenomena which are correlated with gravity sensing, but

it is not yet known whether these are direct measures of gravity sensing or whether they are epiphenomena.

Two groups have measured depolarization of the membrane potential of cells in gravitropically responsive tissue. Behrens et al. (1985) measured depolarization of statocytes with a time scale closely corresponding to the presentation time. Ishikawa et al (1987) measured depolarization in cortical cells in the elongating zone of bean roots in much shorter times than the presentation time. The latter is rather curious because the electrical response preceded any other detectable response, and certainly preceded growth responses expected in the elongating zone. Further the cortical cells appear to have a minor role in gravitropic curvature (Björkman and Cleland, 1988). Dr. Ishikawa has designed a space experiment (Space Biology Experiment, Japanese H2 rocket) in which the depolarization of bean cortical cells is intended to detect gravity sensing.

Making intracellular impalements into specific plant cells is technically quite difficult and is not amenable to automation. Using this technique would require a large time investment on the part of a specifically trained payload specialist. In Dr. Ishikawa's experiment, the sample is placed in the apparatus before launch and the equipment is manipulated remotely from earth.

Two groups have also used a vibrating probe to measure changes in ionic currents around the gravity sensitive tissue. Behrens et al. (1982) made measurements indicating that currents were sensitive to changes in the gravity vector. Björkman and Leopold (1987a) made further investigations and found that the change in ionic current commenced coincident with the presentation time. They also found that the current was sensitive to calmodulin inhibitors (Björkman and Leopold, 1987b). Other data indicate that calmodulin is required to change diageotropism to orthogeotropism, but that it is not directly involved in gravity perception. Hence, the current is either a measure of transduction of perception to growth or that it is an epiphenomenon.

The vibrating probe would be somewhat easier to adapt to space conditions than the intracellular microelectrode because it can be positioned with less precision. However, it is subject to more experimental artifacts, so the training and effort required of a payload specialist is similar.

Ideally, a non-invasive technique would be best suited if an appropriate measure could be developed. External electrodes have been used for many years to measure the so-called geoelectric effect which has many guises, many of which are experimental artifacts. Nevertheless, tissue-level electrical responses of plants to gravity may be detectable with affixed electrodes. Another approach which I have not explored but which may be considered is Magnetic Resonance Imaging to detect consequences of altered electrical fields in the gravisensitive tissue.

Even if electrical measurements in fact detect epiphenomena, those which are strictly consequences of gravity perception may still be useful. A particular issue for which they may be used is to determine whether a gravisensitive tissue in its preferred orientation generates no signal or an equilateral signal. There is no way to test that at present, but it could be done by simply comparing the signal generated in a tissue in its preferred orientation at 1g and that at micro-g. Then these could be related to earth measurements of the intensity and distribution of signal when the tissue is gravistimulated.

At present there is no simple or unequivocal way to specifically measure gravity perception by plants. There is reason to expect that a fully-automated system could be developed based on electrical consequences of gravity

perception with non-invasive techniques (fluorimetry or magnetic resonance) being the most promising. In the meantime, there are microgravity experiments which can be done to make inferences about gravity perception. These are necessary for full benefit to be gained from easier indirect measurements in the future.

CELL CULTURE

Another issue which concerns gravity sensing by cells in space is whether the presence of gravity is required for normal development. A different environmental stimulus which greatly affects development is light, with photomorphogenesis being regulated at very low light doses. Is there an analogous requirement for low doses of gravity? If so, it could be useful to be able to measure whether the cells are detecting gravity even when gravitropism is not an issue.

However, it seems unlikely that gravity serves as such a developmental cue because it is constant during development. In contrast, post-germination growth is usually in the absence of light which produces etiolated plants. These are well suited to growth underground and the response is therefore adaptive.

On the other hand, whereas plants have evolved with gravity present, do plants use it to perform work during development? The most likely process would be mitosis, because the mitotic apparatus is large enough to be significantly affected by gravity. Experiments with laser surgery on the mitotic spindle suggest, however, that the forces applied by the spindle are far greater than gravity.

On multicellular structures, gravity clearly has important mechanical consequences, but these can largely be grouped with thigmomorphogenesis. For example, the compression of a stem by the rest of the plant above it is essentially the same as the compression caused by wind moving the top of the plant about.

In a solution culture, the uptake of nutrients from the medium in a stationary flask in micro-g may be limited because there is no convection of the medium to accelerate diffusion. This response is interesting, but it is not a cellular response to microgravity.

Thus the effect of microgravity on cultured cells is likely to be by large-scale physical events rather than by gravity sensing in the cultured cells. I do not expect that it will be necessary to determine whether individual cultured cells perceive gravity unless cells grow abnormally even after the obvious microgravity effects on the culture as a whole can be ruled out the problem.

References

- Behrens, H.M., M.H. Weisenseel and A. Sievers. 1982. Rapid changes in the pattern of electric current around the root tip of Lepidium sativum L. following gravistimulation. *Plant Physiol.* 70:1079-1083
- Behrens, H.M., D. Gradmann and A. Sievers. 1985. Membrane-potential responses following gravistimulation in roots of Lepidium sativum L. *Planta* 163:463-472

- Björkman, T. and R.E. Cleland. 1988. The role of the epidermis and cortex in gravitropic curvature of maize roots. *Planta*. 176:513-518.
- Björkman, T. and A.C. Leopold. 1987a. An electric current associated with gravity sensing in maize roots. *Plant Physiol.* 84:841-846
- Björkman, T. and A.C. Leopold. 1987b. Effect of inhibitors of auxin transport and of calmodulin of a gravisensing dependent current in maize roots. *Plant Physiol.* 84:847-850
- Ishikawa, H., N. Sawada, K. Tanaka, E. Ohta and M. Sakata. 1985. Proc. Second Space Util. Symp. ISAS, Tokyo. pp. 80-83
- Johnsson, A. 1965. Investigations of the reciprocity rule by means of geotropic and geoelectric measurements. *Physiol. Plant.* 18:945-967